

Amendments to the Claims

Claims 1-7 and 17-47 were previously cancelled. With this amendment, please amend claims 8, 48, 55, and 60; cancel claim 10; and add new claims 70-75, as indicated below:

Claims 1-7. Cancelled.

Claim 8. (currently amended) A method of directing differentiation of human embryonic cells to a specific cell type, comprising:

- a. ~~permitting a population of human embryonic stem cells to form embryoid bodies *in vitro*~~ obtaining a hES cell line from inner mass-stage human embryos;
- b. chemically dissociating the human embryonic stem cells from said cell line;
- c. aggregating the hES cells in suspension, without adherence to the culture vessel, to form embryoid bodies and initial differentiation of the aggregated hES in the embryoid bodies;
- ~~[[b]]~~d. dissociating the embryoid bodies to provide dissociated embryonic cells;
- ~~[[c]]~~e. culturing said dissociated embryonic cells as a monolayer; and
- f. ~~exposing said dissociated embryonic cells derived from the embryoid bodies~~ monolayer to at least one exogenous factor for an effective period of time; ~~and~~
- ~~d. — causing directed~~ to direct differentiation of said dissociated embryonic cells to form the specific cell type comprising a marker for terminally differentiated cells of the specific cell type.

Claim 9. (original) A method according to claim 8, wherein the embryoid bodies are formed in a suspension culture.

Claim 10. (cancel) ~~A method according to claim 8, wherein the dissociated embryonic cells are monolayer cultures.~~

Claim 11. (original) A method according to claim 8, wherein the exogenous factor is a growth factor.

Claim 12. (original) A method according to claim 8, wherein the exogenous factor is an interleukin.

Claim 13. (original) A method according to claim 11, wherein the exogenous factor is nerve growth factor.

Claim 14. (original) A method according to claim 8, wherein the exogenous factor is retinoic acid.

Claim 15. (original) A method according to claim 8, wherein the differentiated cells are neuronal cell type.

Claim 16. (original) A method according to claim 15, wherein the differentiated cells have neuronal processes.

Claims 17-47. Cancelled.

Claim 48. (currently amended) A method of directing differentiation of human embryonic cells to human ectoderm cells, comprising:

a. ~~permitting a population of human embryonic stem cells to form embryoid bodies in vitro~~ obtaining a hES cell line from inner mass-stage human embryos;

b. chemically dissociating the human embryonic stem cells from said cell line;

c. aggregating the hES cells in suspension, without adherence to the culture vessel, to form embryoid bodies and initial differentiation of the aggregated hES in the embryoid bodies;

[[b]]d. dissociating the embryoid bodies to provide dissociated embryonic cells;

~~[[c]]~~e. culturing said dissociated embryonic cells as a monolayer; and
f. exposing said ~~dissociated embryonic cells derived from the embryoid~~
~~bodies~~ monolayer to at least one exogenous factor for an effective period of time; and
d. ~~causing directed~~ to direct differentiation of said dissociated embryonic
cells to form human ectoderm cells comprising a marker for terminally
differentiated human ectoderm cells.

Claim 49. (withdrawn) A method according to claim 48, wherein, in causing, said
embryonic cells form human epidermal skin cells.

Claim 50. (currently amended) A method according to claim 49, wherein, in exposing,
the at least one exogenous factor includes EGF.

Claim 51. (previously presented) A method according to claim 48, wherein, in causing,
said embryonic cells form human brain cells.

Claim 52. (previously presented) A method according to claim 51, wherein, in exposing,
the at least one exogenous factor includes at least one of RA and NGF.

Claim 53. (withdrawn) A method according to claim 48, wherein, in causing, said
embryonic cells form human adrenal cells.

Claim 54. (previously presented) A method according to claim 53, wherein, in exposing,
the at least one exogenous factor includes RA.

Claim 55. (currently amended) A method of directing differentiation of human
embryonic cells to human endoderm cells, comprising:

- a. ~~permitting a population of human embryonic stem cells to form embryoid~~
~~bodies in vitro~~ obtaining a hES cell line from inner mass-stage human embryos;
- b. chemically dissociating the human embryonic stem cells from said cell
line;

c. aggregating the hES cells in suspension, without adherence to the culture vessel, to form embryoid bodies and initial differentiation of the aggregated hES in the embryoid bodies;

[[b]]d. dissociating the embryoid bodies to provide dissociated embryonic cells;

[[c]]e. culturing said dissociated embryonic cells as a monolayer; and

f. exposing said ~~dissociated embryonic cells derived from the embryoid bodies~~ monolayer to at least one exogenous factor for an effective period of time; ~~and~~

d. ~~causing directed~~ to direct differentiation of said dissociated embryonic cells to form human endoderm cells comprising a marker for terminally differentiated human endoderm cells.

Claim 56. (withdrawn) A method according to claim 55, wherein, in causing, said embryonic cells form human liver cells.

Claim 57. (previously presented) A method according to claim 56, wherein, in exposing, the at least one exogenous factor includes at least one of HGF and NGF.

Claim 58. (withdrawn) A method according to claim 55, wherein, in causing, said embryonic cells form human pancreatic cells.

Claim 59. (previously presented) A method according to claim 58, wherein, in exposing, the at least one exogenous factor includes at least one of HGF and NGF.

Claim 60. (currently amended) A method of directing differentiation of human embryonic cells to human mesoderm cells, comprising:

a. ~~permitting a population of human embryonic stem cells to form embryoid bodies in vitro~~ obtaining a hES cell line from inner mass-stage human embryos;

b. chemically dissociating the human embryonic stem cells from said cell line;

c. aggregating the hES cells in suspension, without adherence to the culture vessel, to form embryoid bodies and initial differentiation of the aggregated hES in the embryoid bodies;

[[b]]d. dissociating the embryoid bodies to provide dissociated embryonic cells;

[[c]]e. culturing said dissociated embryonic cells as a monolayer; and

f. exposing said dissociated embryonic cells derived from the embryoid bodies monolayer to at least one exogenous factor for an effective period of time; and

d. causing directed to direct differentiation of said dissociated embryonic cells to form human mesoderm cells comprising a marker for terminally differentiated human mesoderm cells.

Claim 61. (withdrawn) A method according to claim 60, wherein, in causing, said embryonic cells form human chondrocytes.

Claim 62. (previously presented) A method according to claim 61, wherein, in exposing, the at least one exogenous factor includes BMP-4.

Claim 63. (withdrawn) A method according to claim 60, wherein, in causing, said embryonic cells form human kidney cells.

Claim 64. (withdrawn) A method according to claim 60, wherein, in causing, said embryonic cells form human Mullerian duct cells.

Claim 65. (previously presented) A method according to claim 60, wherein, in causing, said embryonic cells form human blood cells.

Claim 66. (withdrawn) A method according to claim 60, wherein, in causing, said embryonic cells form human heart muscle cells.

Claim 67. (previously presented) A method according to claim 66, wherein, in exposing, the at least one exogenous factor includes at least one of TGF- β and activin-A.

Claim 68. (withdrawn) A method according to claim 60, wherein, in causing, said embryonic cells form human skeletal muscle cells.

Claim 69. (previously presented) A method according to claim 68, wherein, in exposing, the at least one exogenous factor includes at least one of TGF- β and activin-A.

70. (new) A method of directing differentiation of human embryonic cells to human neuronal cells comprising:

- a. obtaining a hES cell line from inner mass-stage human embryos;
- b. chemically dissociating the human embryonic stem cells from said cell line;
- c. aggregating the hES cells in suspension, without adherence to the culture vessel, to form embryoid bodies and initial differentiation of the aggregated hES in the embryoid bodies;
- d. dissociating the embryoid bodies to provide dissociated embryonic cells;
- e. culturing said dissociated embryonic cells as a monolayer; and
- f. exposing said monolayer to at least one exogenous factor for an effective period of time to direct differentiation of said dissociated embryonic cells to form the neuronal cells.

71. (new) A method of directing differentiation of human embryonic cells to human muscle cells comprising:

- a. obtaining a hES cell line from inner mass-stage human embryos;
- b. chemically dissociating the human embryonic stem cells from said cell line;
- c. aggregating the hES cells in suspension, without adherence to the culture vessel, to form embryoid bodies and initial differentiation of the aggregated hES in the embryoid bodies;
- d. dissociating the embryoid bodies to provide dissociated embryonic cells;
- e. culturing said dissociated embryonic cells as a monolayer; and

f. exposing said monolayer to at least one exogenous factor for an effective period of time to direct differentiation of said dissociated embryonic cells to form the muscle cells.

72. (new) A method according to claim 71, wherein the muscle cells are cardiomyocytes.

73. (new) A method of directing differentiation of human embryonic cells to human pancreatic cells comprising:

- a. obtaining a hES cell line from inner mass-stage human embryos;
- b. chemically dissociating the human embryonic stem cells from said cell line;
- c. aggregating the hES cells in suspension, without adherence to the culture vessel, to form embryoid bodies and initial differentiation of the aggregated hES in the embryoid bodies;
- d. dissociating the embryoid bodies to provide dissociated embryonic cells;
- e. culturing said dissociated embryonic cells as a monolayer; and
- f. exposing said monolayer to at least one exogenous factor for an effective period of time to direct differentiation of said dissociated embryonic cells to form the pancreatic cells.

74. (new) A method of making human embryonic bodies from human embryonic stem cells comprising:

- a. obtaining a hES cell line from inner mass-stage human embryos;
- b. chemically dissociating the human embryonic stem cells from said cell line;
- c. aggregating the hES cells in suspension, without adherence to the culture vessel, so as to form embryoid bodies and initial differentiation of the aggregated hES in the embryoid bodies.

75. (new) A method of making human embryonic cells from human embryonic bodies comprising:

- a. obtaining a hES cell line from inner mass-stage human embryos;
- b. chemically dissociating the human embryonic stem cells from said cell line;
- c. aggregating the hES cells in suspension, without adherence to the culture vessel, to form embryoid bodies and initial differentiation of the aggregated hES in the embryoid bodies;
- d. dissociating the embryoid bodies to provide dissociated embryonic cells; and
- e. culturing said dissociated embryonic cells.